

Proteins of the *Bacillus stearothermophilus* ribosome

A low resolution crystal analysis of protein L30

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Received 30 June 1983

The 5 Å resolution crystal structure analysis of ribosomal protein L30 from *Bacillus stearothermophilus* is described. The molecule is shown to be compact and extend to about 25–30 Å in each dimension.

Ribosomal protein

Thermophile

Crystal structure

1. INTRODUCTION

The amino acid sequence of L30 from *Bacillus stearothermophilus* has been determined within this institute [1], as has that of the homologous protein from *Escherichia coli* [2]. The two sequences have identical amino acids at 53% of the positions. This well-established homology will allow us to relate experimental results on the *E. coli* protein to our 3-D structure.

L30 was extracted from *B. stearothermophilus* under non-denaturing conditions and the NMR spectrum indicates a well-ordered structure for the isolated protein [3]. CD spectra show an α -helix content of 35% for the *E. coli* and 38% for the *B. stearothermophilus* protein (unpublished). The protein contains 62 amino acids, but lacks cysteine, tyrosine, tryptophan and phenylalanine [1].

Here, we describe the structure of ribosomal protein L30 from *B. stearothermophilus* as determined by X-ray crystallography at a nominal resolution of 5 Å. The crystallisation of this protein has been reported in [4]. This is the first report of a crystal structure of an intact ribosomal protein.

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2. MATERIALS AND METHODS

2.1. Protein extraction and crystallisation

The protein was isolated under mild conditions avoiding acetic acid and urea [3] and crystallised as in [4] by the hanging drop vapour diffusion technique.

2.2. X-ray diffraction

Diffraction data were recorded on an Enraf-Nonius Arndt-Wonacott oscillation camera with CuK α X-rays produced by a Seifert stationary anode operating with a fine focus tube at 40 kV and 30 mA.

3. RESULTS AND DISCUSSION

Crystals of L30 grow optimally at pH 8.4 in 0.1 M Tris-HCl buffer, 3.85 M (NH₄)₂SO₄ and at protein concentrations of 4–10 mg/ml [4]. The crystals are well-ordered and diffract to roughly 2.5 Å resolution. The space group is P4₃2₁2, the enantiomorph having been defined from the anomalous scattering of the heavy-atom derivatives. The cell dimensions are $a = b = 46.3$ Å, $c = 61.4$ Å, giving a unit cell volume of 131 623 Å³. Assumption of 1 molecule/asymmetric

Table 1
Summary of the 5 Å X-ray data for L30

Crystal	Heavy atom reagent (mM)	Soak time (days)	R_{sym}	Δ_{iso}
1. Native protein	—	—	0.085	—
2. $\text{Pt}(\text{NH}_3)_2\text{Cl}_2$ derivative	1	1	0.045	0.227
3. PtCl_4 derivative	1	1	0.052	0.245
4. $\text{KAu}(\text{CN})_2$ derivative	1	3	0.057	0.252

R_{sym} is defined as:

$$\frac{\sum_h \sum_i |I_{ih} - \bar{I}|}{\sum_h \sum_i I_{ih}}$$

where:

\bar{I} = the mean intensity of i equivalent reflections with indices given by h

Δ_{iso} is the mean fractional isomorphous difference summed over all reflections

unit gives a volume of the asymmetric unit/ M_r -value of 2.33 Å³/dalton [4].

Oscillation data have been processed and evaluated to a nominal resolution of 5 Å for rapid screening of the heavy atom derivatives. The data are summarised in table 1. Difference Patterson

syntheses have been calculated for the three derivatives. The two platinum derivatives proved to be almost identical, with a single binding site (table 2). The least-squares refinement of these gave an excellent minimum, and the derivatives clearly provide high quality phase information. The gold derivative was interpreted in terms of two binding sites (table 2). Unfortunately the major site lies extremely close to the 2-fold rotation axis in the unit cell and the minor site also has a closely similar value for the x and y coordinate. The 'special positions' of these gold sites together with (and indeed partly causing) the relatively poor refinement criteria for this derivative (table 2) mean that the gold contributes very weakly to the phasing of the protein, and will not be useful at high resolution.

Phases for the native protein were calculated to 5 Å from the 3 derivatives, the dominance of the 2 almost identical platinum compounds over the gold giving essentially a single isomorphous plus anomalous phase determination. Nevertheless at this resolution the phases are well defined with a mean figure of merit of 0.84 (table 2).

An electron density map was calculated with these phases. A superposition of 7 sections, from $Z = 0.1$ – 0.25 , of this synthesis is shown in fig.1. There is good distinction between the electron dense protein regions and the clear solvent regions in this synthesis. The molecular boundary is clearly

Table 2
The parameters for the least-squares refinement of the heavy atom derivatives

Derivative	Occupancy	x	y	z	B	f_H	E	R_c
1. $\text{Pt}(\text{NH}_3)_2\text{Cl}_2$	1.600	0.148	0.475	0.422	15	250	118	0.345
2. PtCl_4	1.700	0.142	0.477	0.426	15	276	113	0.288
3. $\text{KAu}(\text{CN})_2$	1.229 0.572	0.148 0.068	0.173 0.032	0.011 0.308	15 15	278	183	0.474

The occupancies are on an arbitrary scale, and the temperature (B) values have not been refined. f_H is the mean calculated heavy atom contribution, E the RMS lack of closure error. R_c is the R factor for the centric terms during the refinement:

$$R = \frac{\sum_h |F_{PH} - F_P| - f_H}{\sum_L |F_{PH} - F_P|}$$

where:

F_P = the structure factor amplitude of the native protein;

F_{PH} = that of the heavy atom derivative

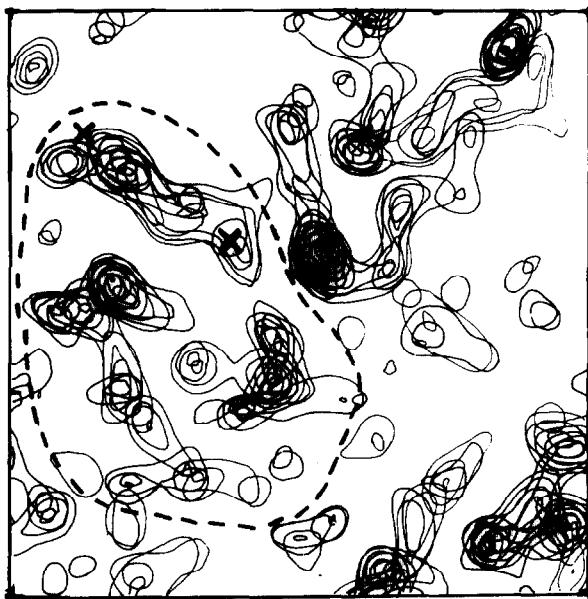


Fig.1. A superposition of 7 sections from $Z = 0.1-0.25$ of the 5 Å electron density synthesis of protein L30. The dotted line indicates the molecular boundary. The two crosses on the left of the molecule show one of the two rod-like, helical, features. A complete unit cell in the x and y directions is shown in these sections, with the origin of the cell at the top left.

defined when the map is viewed in three dimensions, and is shown in the figure. The molecule is compact, extending about 25–30 Å in each dimen-

sion. Two electron-dense rod-like features (one is indicated in the figure) are almost certainly α -helical segments of the protein chain. Their lengths suggest they accommodate about 20 amino acids in all, in keeping with our estimates from the CD spectra of L30 which suggested roughly 35% of the 62 residues were α -helical (unpublished).

We are currently evaluating native and platinum data to a resolution of 2.5 Å, and also searching for further isomorphous derivatives. We hope to calculate a high resolution image of the molecule in the near future.

ACKNOWLEDGEMENTS

We thank Professor H.-G. Wittmann for his encouragement of these studies. The work has been supported by the Deutsche Forschungsgemeinschaft (SFB9).

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